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Date: July 11, 2001

18

By:

Kay L. Gaviglio

PATENT

Docket No. GC450-D1-US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of

De Buyl et al.

Serial No.: Unknown

Filed: Herewith

For: **XYLANASE, MICORORGANISMS
PRODUCING IT, DNA MOLECULES,
METHODS FOR PREPARING THIS
XYLANASE AND USES OF THE LATTER**

Group Art Unit: Unknown

Examiner: Unknown

PRELIMINARY AMENDMENT

Assistant Director of Patents
Washington, DC 20231

Sir:

This application is being filed as a divisional application of application serial number 08/470,953, filed June 6, 1995. Prior to the initial examination of the application, please enter the following preliminary amendment.

IN THE SPECIFICATION:

Please substitute the abstract of the disclosure with the following clean copy. The new abstract is also submitted on a separate sheet attached hereto along with a marked up version.

-- The invention relates to an isolated and purified culture of Bacillus sp. strain 720/1 (LMG P-14798); to xylanases obtained from this strain and xylanases obtained from

derivatives and mutants of strain 720/1. The invention also relates to a DNA molecule encoding a xylanase and to expression vectors or integration vectors containing the DNA molecule. This invention also relates to transformed host strains comprising the DNA molecule encoding the xylanase. - -

IN THE CLAIMS:

Please cancel claims 1 - 6 and 19 - 24.

Replace claims 8 - 12, 15 - 18 and 25 - 30 with the following clean copy of said amended claims. A marked-up version of the amended claims is provided herein on a separate sheet.

8.(Amended) An isolated DNA molecule comprising the nucleotide sequence illustrated in SEQ ID NO: 1 which codes for the mature xylanase of *Bacillus* sp. 720/1 (LMG P-14798) or a modified sequence derived from this sequence.

9.(Amended) An isolated DNA molecule comprising the nucleotide sequence illustrated in SEQ ID NO: 4 which codes for the *Bacillus* sp 720/1 xylanase precursor or a modified sequence derived from this sequence.

10.(Amended) An isolated DNA molecule comprising the *Bacillus* sp 720/2 xylanase gene as illustrated in SEQ ID NO: 10.

11.(Amended) The isolated DNA molecule according to Claim 32, wherein said DNA molecule comprises a promoter having the sequence illustrated in SEQ ID NO: 26 derived from the gene which codes for *Bacillus pumilus* B12 PRL xylanase and a presequence illustrated in SEQ ID NO: 27 which codes for the signal peptide of *Bacillus pumilus* PRL B12 xylanase.

12.(Amended) An expression vector or chromosomal integration vector comprising the DNA molecule according to Claim 8, 9, 10, 11, 32 or 33.

15.(Amended) A transformed strain comprising the DNA molecule according to Claim 8, 9, 10, 11, 32 or 33.

16.(Amended) A transformed strain comprising the expression vector or chromosomal integration vector according to Claim 12, 13 or 14.

17.(Amended) The transformed strain according to Claim 15 or 16, wherein said strain is a *Bacillus* strain.

18.(Amended) The transformed strain according to Claim 17, wherein said strain is a *Bacillus licheniformis* or *Bacillus pumilus* strain.

25.(Amended) A promoter comprising the sequence illustrated in SEQ ID NO: 26, wherein said promoter is derived from the gene which codes for *Bacillus pumilus* PRL B12 xylanase.

26.(Amended) A presequence comprising the sequence illustrated in SEQ ID NO: 27 which codes for the signal peptide of *Bacillus pumilus* PRL B12 xylanase.

27.(Amended) An expression system which can be used for the production of a polypeptide, comprising:

- a) a promoter comprising the sequence illustrated in SEQ ID NO: 26 derived from the gene which codes for *Bacillus pumilus* PRL B12 xylanase,
- b) a sequence coding for a signal peptide, and
- c) a sequence encoding the polypeptide.

28.(Amended) An expression system which can be used for the production of a polypeptide comprising:

- a) a promoter;
- b) a presequence having the sequence illustrated in SEQ ID NO: 27 which codes for the signal peptide of *Bacillus pumilus* PRL B12 xylanase, and
- c) a sequence encoding the polypeptide.

29.(Amended) An expression system which can be used for the production of a polypeptide comprising:

- a) a promoter having the sequence illustrated in SEQ ID NO: 26 derived from the gene which codes for *Bacillus pumilus* PRL B12 xylanase;

b) a presequence having the sequence illustrated in SEQ ID NO: 27 which codes for the signal peptide of *Bacillus pumilus* PRL B12 xylanase,

c) a sequence encoding the polypeptide, and

d) a terminator sequence.

30. The expression system according to Claim 27, 28 or 29, wherein the polypeptide corresponds to the nucleotide sequence of SEQ ID NO: 1 which codes for *Bacillus* sp. 720/1 xylanase.

Please add the following new claims:

31. The isolated culture of claim 7, wherein the culture is *Bacillus* sp 720/1 (LMG P-14798).

32. The isolated DNA molecule of claim 8, wherein said DNA molecule comprises SEQ ID NO: 1.

33. The isolated DNA molecule of claim 9, wherein said DNA molecule comprises SEQ ID NO: 4.

34. The expression system according to Claim 27, 28 or 29, wherein the polypeptide is selected from the group consisting of a protease, a lipase, a xylanase, a cellulase, an amylase and a pullulanase.

35. The expression system according to Claim 34, wherein said polypeptide is a xylanase.

REMARKS

The parent application, serial number 08/470,953 filed June 6, 1995 was subject to a restriction requirement in paper number 9, dated March 20, 1997. The inventions were designated as Group I, claims 1 - 6 and 19 - 24 drawn to an enzyme and methods of using the enzyme, classified in class 435, subclass 183 and Group II, claims 7 - 18 and 25 - 30 drawn to isolated DNA sequences, expression vectors, transformed bacterial host cells and methods of expressing a protein, classified in class 435, subclass 320.1 and class 435, subclass 252.2. In the parent application, Applicants elected the invention denominated as Group I. This instant divisional application is directed to the invention denominated as Group II.

Claims 7 - 18 and 25 - 35 are pending in the application. Claims 1 - 6 and 19 - 24 have been canceled. Claims 8 - 12, 15 - 18 and 25 - 30 have been amended and claims 31 - 35 are added by the instant amendment.

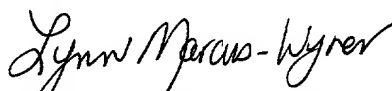
Claims 8 - 12 and 16 - 25 have been amended to merely comply with standard claim drafting protocols.

New claims 31 - 33 find support in the original claims. Claim 34 is a multiple dependent claim and recites different enzyme classes as the polypeptide. Support may be found at least at page 11, lines 31 - 39 of the specification. Claim 35 is dependent on claim 34 and defines the enzyme as a xylanase.

Applicants have additionally provided a new abstract of the disclosure.

Applicants kindly solicit the allowance of claims 7 - 18 and 25 - 35.

Respectfully submitted,



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Date: July 16, 2001

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MARKED-UP VERSION OF THE ABSTRACT

The invention relates to [a xylanase originating from a *Bacillus* strain. This xylanase is active over a wide range of acid and basic pH.

The invention also relates to new strains of microorganisms producing this xylanase and to methods for preparing this xylanase.] an isolated and purified culture of *Bacillus* sp. strain 720/1 (LMG P-14798); to xylanases obtained from this strain and xylanases obtained from derivatives and mutants of strain 720/1.

The invention also relates to a DNA molecule encoding a xylanase and to [an] expression vectors or [an] integration vectors containing the DNA molecule.

This invention also relates to [uses of the latter and to composition containing it. Figure 2] transformed host strains comprising the DNA molecule encoding the xylanase.

Clean Copy of the Abstract

The invention relates to an isolated and purified culture of *Bacillus* sp. strain 720/1 (LMG P-14798); to xylanases obtained from this strain and xylanases obtained from derivatives and mutants of strain 720/1. The invention also relates to a DNA molecule encoding a xylanase and to expression vectors or integration vectors containing the DNA molecule. This invention also relates to transformed host strains comprising the DNA molecule encoding the xylanase.

MARKED-UP VERSION OF AMENDED CLAIMS

8.(Amended) An isolated DNA molecule comprising the nucleotide sequence [(SEQ ID NO: 1)] illustrated in SEQ ID NO: 1 which codes for the mature xylanase of Bacillus sp. 720/1 (LMG P-14798) or a modified sequence derived from this sequence.

9.(Amended) An isolated DNA molecule [according to Claim 8, characterized in that it comprises] comprising the nucleotide sequence [(SEQ ID NO: 4)] illustrated in SEQ ID NO: 4 which codes for the Bacillus sp 720/1 xylanase precursor or a modified sequence derived from this sequence.

10.(Amended) An isolated DNA molecule [according to Claim 8 or 9, characterized in that it comprises] comprising the [entire] Bacillus sp 720/2 xylanase gene [(SEQ ID NO:10)] as illustrated in SEQ ID NO: 10.

11.(Amended) The isolated DNA molecule according to Claim [8, characterized in that it] 32, wherein said DNA molecule comprises [the] a promoter [(SEQ ID NO: 26)] having the sequence illustrated in SEQ ID NO: 26 derived from the gene which codes for Bacillus pumilus PRL B12 xylanase[, the] and a presequence [(SEQ ID NO:27)] illustrated in SEQ ID NO: 27 which codes for the signal peptide of Bacillus pumilus PRL B12 xylanase [and the nucleotide sequence (SEQ ID NO: 1) which codes for Bacillus sp. 720/1 xylanase].

12.(Amended) [Expression] An expression vector or chromosomal integration vector [containing] comprising the DNA molecule according to Claim 8, 9, 10, [or] 11, 32 or 33.

15.(Amended) [Transformed] A transformed strain comprising the DNA molecule according to Claim 8, 9, 10, [or] 11, 32 or 33.

16.(Amended) [Transformed] A transformed strain comprising the expression vector or chromosomal integration vector according to Claim 12, 13 or 14.

17.(Amended) [Transformed] The transformed strain according to Claim 15 or 16, [characterized in that it] wherein said strain is a Bacillus strain.

18.(Amended) [Transformed] The transformed strain according to Claim 17, [characterized in that it] wherein said strain is a Bacillus licheniformis or Bacillus pumilus strain.

25.(Amended) [Promoter (SEQ ID NO: 26)] A promoter comprising the sequence illustrated in SEQ ID NO: 26, wherein said promoter is derived from the gene which codes for Bacillus pumilus PRL B12 xylanase.

26.(Amended) [Presequence (SEQ ID NO:27)] A presequence comprising the sequence illustrated in SEQ ID NO: 27 which codes for the signal peptide of Bacillus pumilus PRL B12 xylanase.

27.(Amended) [Expression] An expression system which can be used for the production of a polypeptide, [characterized in that it comprises] comprising:

a) a promoter [the sequence of the promoter (SEQ ID NO: 26)] comprising the sequence illustrated in SEQ ID NO: 26 derived from the gene which codes for Bacillus pumilus PRL B12 xylanase,

b) a sequence coding for a signal peptide, and

[the] c) a sequence encoding [of] the polypeptide [of interest].

28.(Amended) [Expression] An expression system which can be used for the production of a polypeptide[, characterized in that it comprises] comprising:

[the sequence of a promoter;] a) a promoter;

[the] b) a presequence [(SEQ ID NO: 27)] having the sequence illustrated in SEQ ID NO: 27 which codes for the signal peptide of Bacillus pumilus PRL B12 xylanase, and

[the] c) a sequence encoding [of] the polypeptide [of interest].

29.(Amended) [Expression] An expression system which can be used for the production of a polypeptide[, characterized in that it comprises] comprising:

[the sequence of the] a) a promoter [(SEQ ID NO: 26)] having the sequence illustrated in SEQ ID NO: 26 derived from the gene which codes for Bacillus pumilus PRL B12 xylanase;

[the] b) a presequence [(SEQ ID NO: 27)] having the sequence illustrated in SEQ ID NO: 27 which codes for the signal peptide of *Bacillus pumilus* PRL B12 xylanase,
[the] c) a sequence encoding [of] the polypeptide [of interest], and [the sequence of] d) a terminator sequence.

30. [Expression] The expression system according to Claim 27, 28 or 29, [characterized in that] wherein [the sequence of] the polypeptide [of interest] corresponds to the nucleotide sequence of [(SEQ ID NO: 1)] which codes for *Bacillus* sp. 720/1 xylanase.